Plasma Thymidine Kinase Activity in Dogs with Lymphoma and Leukemia

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(Received 6 May 1997/Accepted 27 June 1997)

ABSTRACT. Plasma thymidine kinase (TK) activity was evaluated as a plasma marker for canine lymphoma and leukemia. A tentative "cut-off" value was set at 6.0 U/*l* as the upper level of plasma TK based on the mean + 2SD of plasma TK activity in 13 clinically healthy dogs. The levels of plasma TK activity in all of the 20 dogs with lymphoma and leukemia were higher than the cut-off value, whereas those in dogs with lymphoma decreased in parallel with the reduction of the tumor mass after chemotherapy. These findings suggested that estimation of plasma TK activity can be used as a plasma marker for lymphoma and leukemia in the dog. — KEY WORDS: canine, lymphoma, thymidine kinase (TK).

J. Vet. Med. Sci. 59(10): 957–960, 1997

Dogs are frequently the victims of the neoplastic diseases, lymphoma and leukemia. Canine lymphoma and leukemia are consistently fatal diseases, although they can be controlled with combination chemotherapy when diagnosed at early disease stages. Effective chemotherapeutic treatment, therefore, makes the diagnosis and evaluation of the clinical status of dogs with lymphoma and leukemia very important. The diagnosis of lymphoma and leukemia is based on histopathological and cytological findings; as a result, establishing diagnoses in cases from which tissue samples for histopathological examination are not available is a challenge. In these cases, a non-invasive diagnostic procedure can be very helpful.

Serum or plasma markers for tumors, including alpha-fetoprotein [12, 13], carcinoembryonic antigen [10] and several other markers for tumors [1, 4, 5, 17], are very useful diagnostic tools. Furthermore, these serum or plasma markers for tumors aid in estimation of disease prognosis, therapeutic effect and tumor relapse, because the levels of these markers are frequently influenced by the growth of tumor cells.

Thymidine kinase (TK) is a phosphorylase that converts thymidine to thymidine monophosphate. Especially in rapidly proliferating cells, it serves as part of an alternative metabolic pathway for DNA synthesis [2, 3]. Increases in serum TK concentrations occur in human with acute and chronic leukemia [6, 9, 14], Hodgkin's and non-Hodgkin's lymphoma [7, 18] and multiple myeloma [11]. Even though estimation of serum TK activity is used for diagnosis and monitoring in human with lymphoma and leukemia [7], it has to be applied for diagnosis of the corresponding diseases in small animals.

The present study attempted to establish the significance of plasma TK levels in the diagnosis and monitoring of lymphoma and leukemia in dogs.

Plasma samples were obtained from 20 dogs with lymphoma and leukemia at the Veterinary Medical Center, the University of Tokyo, from March 1995 to February 1997. These dogs consisted of 17 cases with multicentric form lymphoma, 1 case with alimentary lymphoma, 1 case with acute myeloid leukemia (monocytic leukemia, M5b) and 1 case with chronic myeloid leukemia. The diagnosis was made by histopathological and cytological findings of the lymph node or bone marrow specimen at biopsy or autopsy. We also obtained plasma samples from 4 dogs with non-hemopoietic neoplasms including mastocytoma, mammary gland tumor, malignant histiocytosis and anal sac gland tumor, and 5 dogs with non-neoplastic leukocytosis (WBC count, 30×10^3–$100 \times 10^3/\mu l$). As a control group, plasma samples from 13 clinically healthy dogs were also used. The clinical data for these dogs are listed in Table 1. Plasma samples before and after treatment with chemotherapy were also obtained from 5 cases with lymphoma.

Plasma samples separated from the heparinized blood of the dogs were stored at −20°C and used for TK activity assay within 3 weeks after collection. Levels of plasma TK activity were estimated with Prolifigen TK Kit "Daiichi" (Daiichi Radioisotope Lab., Tokyo, Japan), which is a radioenzyme assay system employing ^{125}I-iododeoxyuridine (IUdR) as a tracer. ^{125}I-labeled deoxyuridine was added to plasma samples as substrate for TK and then incubated for 4 hr at 37°C. The reaction was stopped by adding ion-exchange resin which separates ^{125}I-IUdR monophosphate from the substrate, and the radioactivity in the samples was estimated in a gamma-counter.

Mean TK activity for dogs with hemopoietic tumors and clinically healthy dogs, and mean TK activity and LDH activity compared using a two sample *t* test. Statistical significance in this paper refers to the 1 per cent level.

Prior to examining the level of plasma TK activity in patients, the level of TK activity in healthy dog was established. Thirteen clinically healthy dogs, we established the upper level of TK activity in normal canine plasma as having a tentative cut-off value of 6.0 U/*l* which was 2SD above the mean value (3.0 U/*l*). The level of plasma TK activity in 20 dogs with lymphoma and leukemia before chemotherapy ranged from 6.8 to 430 U/*l* (Table 1); these values were significantly higher than the cut-off value ($p<0.01$) (Fig. 1). The levels of plasma TK activity in 4

Table 1. Summary of clinical data for 42 dogs

Case No.	Breed	Sex	Age (yrs)	Diagnosis	TK (U/*l*)	LDH (U/*l*)
Lymphoma and leukemia						
1	Pomeranian	F[a)]	5	Lymphoma	260	583
2	Miniature Dachshund	M[b)]	4	Lymphoma	65	251
3	Maltese	M	8	Lymphoma	380	711
4	West Highland White Terrier	M	9	Lymphoma	110	216
5	Golden Retriever	M	4	Lymphoma	17	214
6	Mixed	M	15	Lymphoma	430	401
7	Beagle	F	3	Lymphoma	200	281
8	Labrador Retriever	M	5	Lymphoma	370	421
9	Siberian Husky	M	4	Lymphoma	7.2	125
10	Shin Tzu	M	12	Lymphoma	21	148
11	Mixed	M*	7	Lymphoma	12	578
12	Mixed	M*	6	Lymphoma	160	376
13	Shih Tzu	M	7	Lymphoma	70	242
14	Beagle	F*	7	Lymphoma	353	616
15	Beagle	M*	11	Lymphoma	23	233
16	Mixed	F*	13	Lymphoma	6.8	128
17	Maltese	M	4	Lymphoma	264	321
18	Shih Tzu	F	7	Lymphoma	173	387
19	Mixed	M	9	Acute myeloid leukemia (M5b)	160	578
20	Irish Setter	M	8	Chronic myeloid leukemia	25	460
Non-hemopoietic tumor						
21	Mixed	M	11	Mastocytoma	0.9	NT[c)]
22	Mixed	F	13	Mammary gland tumor	2.4	NT
23	Mixed	F*	16	Malignant histiocytosis	4.6	NT
24	Mixed	F*	9	Anal sac gland tumor	1.3	NT
Non-neoplastic leukocytosis						
25	Mixed	F	14	Diabetes mellitus	0.7	NT
26	American Cocker Spaniel	M	2	Seborrheic dermatitis	0.8	NT
27	Yorkshire Terrier	F	13	Pyometra	25	NT
28	Yorkshire Terrier	F*	16	Upper respiratory infection	0.9	NT
29	Miniature Schnauzer	F*	4	Tongue necrosis	13	NT
Clinically healthy						
30	Mixed	M	8	Healthy	5.8	NT
31	Mixed	F	9	Healthy	0.8	NT
32	Labrador Retriever	M	5	Healthy	2	NT
33	Beagle	M	1	Healthy	4.2	NT
34	Beagle	M	1	Healthy	3.9	NT
35	Beagle	F	1	Healthy	3.4	NT
36	Beagle	F	1	Healthy	3	NT
37	Beagle	M	6	Healthy	3.1	NT
38	Beagle	M	4	Healthy	4.9	NT
39	Beagle	F	2	Healthy	2.1	NT
40	Beagle	F	1	Healthy	2	NT
41	Beagle	F	4	Healthy	0.9	NT
42	Beagle	F	2	Healthy	2.7	NT

a)Female, b)Male, c)Not tested, *:Neutered.

dogs with non-hemopoietic tumors including mastocytoma, mammary gland tumor, malignant histiocytosis and anal sac gland tumor were below the normal cut-off value (Fig. 1). Levels of plasma TK activity in 5 cases with inflammatory leukocytosis (30×10^3–$100 \times 10^3/\mu l$) accompanied with diabetes mellitus, seborrheic dermatitis, pyometra, upper respiratory infection and tongue necrosis were also examined. The levels of plasma TK activity in 3 cases (cases 25, 26 and 28) were very low, and those in 2 cases (case 27 and 29) were slightly higher than the cut-off value (Fig. 1). After a decrease in the leukocyte counts, however, the levels of plasma TK activity in cases 27 and 29 later decreased to levels below the cut-off value.

Plasma LDH activity in dogs with lymphoma and leukemia ranged from 125 to 711 U/*l* (371 ± 184 U/*l*). As shown in Fig. 2, a significant correlation (r=0.642, p<0.01) was observed between the TK activity and LDH activity in these plasma samples. Plasma TK activity were particularly

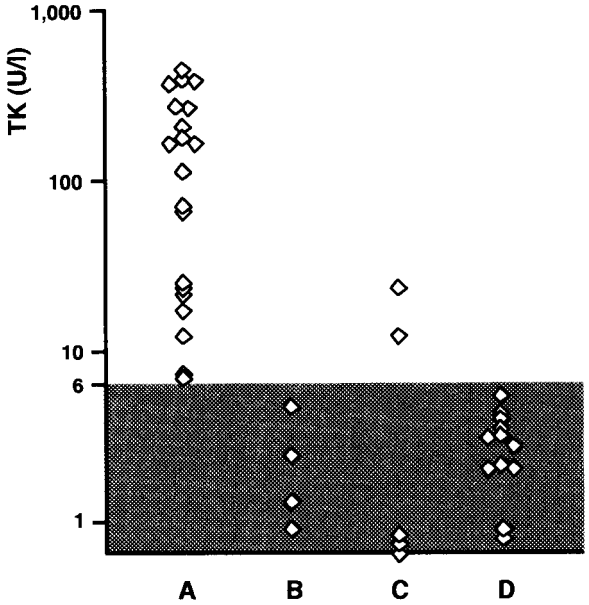

Fig. 1. Levels of plasma TK activity in 42 dogs; 20 dogs with lymphoma and leukemia (A), 4 dogs with non-hemopoietic tumors (B), 5 dogs with non-neoplastic leukocytosis (C) and 13 clinically healthy dogs (D). The shadowed region indicates the normal range of plasma TK activity (less than 6.0 U/*l*).

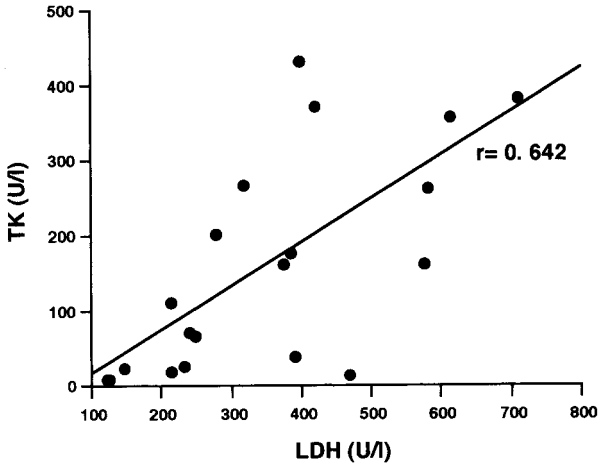

Fig. 2. Correlation between the plasma levels of TK and LDH activity in plasma from 20 dogs with lymphoma and leukemia. r=0.642.

high (> 100 U/*l*) in 6 lymphoma and leukemia cases with high levels of plasma LDH activity (> 400 U/*l*). In 7 lymphoma cases (case 2, 4, 5, 7, 10, 13 and 16), plasma LDH activity were within the normal range (< 350 U/*l*), however, the levels of plasma LDH activity were distinctly higher than the cut-off value ($p<0.01$) (6.0 U/*l*).

Plasma TK activity were measured in 5 dogs with lymphoma (cases 2, 5, 10, 11 and 14) after initiation of chemotherapy. The levels of plasma TK activity in these cases decreased to the levels below the cut-off value in

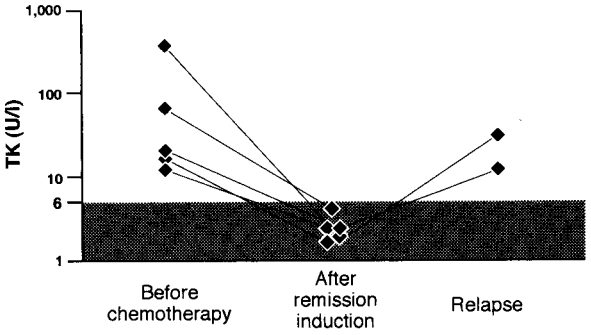

Fig. 3. Changes of plasma TK activity in 5 dogs with lymphoma. TK activity in 5 cases (case 2, 5, 10, 11 and 14) before chemotherapy, after remission induction and at relapse are shown.

parallel with the reduction of the mass volume (Fig. 3). The level of plasma TK activity in cases 5 and 10 was examined when the dog had a relapse of the tumor 10 and 9 months respectively after initiation of chemotherapy, and was found to be re-elevated (31 and 12 *U/l*, respectively) above the cut-off value.

TK is a cellular enzyme that operates in a salvage pathway of DNA synthesis. TK is activated during the G1/S phase of the cell cycle, and its activity in plasma correlates with the proliferation of tumor cells [2]. An improved method for direct quantification of TK activity that uses ^{125}I-IUdR as a substrate [16] is available as a kit from several commercial diagnostic laboratories. For plasma TK assay, heparinized plasma can be stored at −20°C for a long time [16]. Though the cut-off value of normal plasma TK activity in dogs was tentatively set at 6.0 U/*l* in this study, its cut-off value in human was shown to be 5.5 U/*l* [16]. The normal range of the plasma TK activity and its cut-off value in dogs should be fine-tuned by further examination of a large number of canine samples.

In this study, plasma TK activity was elevated in all of the 20 dogs with lymphoma and leukemia before chemotherapy. Though plasma TK activity seemed to be a sensitive plasma marker for lymphoma and leukemia in this study, most of these cases were in advanced stages (IV to V). Estimation of the plasma TK activity in early stages of canine lymphoma and leukemia is needed.

Serum TK activity is sometimes elevated in cases of viral infection, after administration of granulocyte-colony stimulating factor (G-CSF) and in pregnant women [8]. The present study also indicated that plasma TK activity was elevated in two cases with leukocytosis derived from pyometra and tongue necrosis. Although the plasma TK activity in the non-neoplastic diseases were above the cut-off value, they were relatively low in comparison with those in cases with lymphoma and leukemia. However, it should be noted that the plasma TK activity may be elevated due to active proliferation of cells, especially in the hematopoietic and lymphoid systems. As shown in this study, the high levels of plasma TK activity derived from non-neoplastic diseases may decrease to levels below the cut-off value

immediately after the decrease in leukocyte counts.

Plasma LDH activity is often elevated in dogs with lymphoma and leukemia. The level of plasma LDH activity was correlated with that of plasma TK activity in dogs with lymphoma and leukemia in this study. Plasma LDH activity, however, is not a specific marker for lymphoma and leukemia, because it is elevated in a variety of diseases associated with hepatocellular and myocardial damage as well as hemolysis [15]. The plasma LDH activity was not as sensitive a marker as plasma TK activity for lymphoma and leukemia because the plasma LDH activity was not elevated in some cases with lymphoma and leukemia in this study.

The levels of plasma TK activity in 5 dogs with lymphoma decreased in parallel with the reduction of the tumor volume after chemotherapy, indicating that the decrease in plasma TK activity may be an indicator for remission of lymphoma. The level of plasma TK activity were re-elevated at the relapse in two cases with lymphoma in this study. Although we could examine only one case when the dog had a relapse, the re-elevation of the plasma TK activity may be a potential plasma marker for relapse. Eventually, the use of plasma TK activity as a marker for tumor proliferation should be helpful in the control of canine lymphoma and leukemia with chemotherapy.

ACKNOWLEDGEMENT. This study was supported in part by grant from the Ministry of Education, Science, Sports and Culture, Japan.

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